REMARKS

Applicants' remarks to overcome the claim rejection under first paragraph of 35 U.S. C 112

The examiner has acknowledged that the present invention may be practiced analogously for the several antimetabolites disclosed and that, therefore, the number of species within the genus represented by the generic claim should not be at issue. Please see the interview summaries for Telephone Interviews on 10/16/09 and 12/8/09.

However, the examiner had raised the question whether the applicants have reasonably showed the possession of the claimed genus and rejected, for lack of written description to support, claims 51, 54-74 and 76-90 which recite or inherit the limitation, "an oligonucleotide comprising at least two CpG moieties and a **nucleoside antimetabolite** covalently linked to the oligonucleotide."

Applicants respectfully request that this rejection be reconsidered and withdrawn in view of the remarks that follow, scientific citations and the other documentation submitted herewith, which show that the applicants were in possession of the claimed genus, based on their disclosure of a combination of relevant functional characteristics coupled with a correlation between function and structure well known in the prior art

Section 112, paragraph 1 of the Patent Act sets for the written description requirement as follows:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or which it is nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out this invention.

To satisfy the written description requirement, "the applicant does not have to utilize any particular form of disclosure to describe the subject mater claimed, but the description must clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." In re Alton, 76 F.3d 1168, 1172 (Fed. Cir. 1996). In other words, the applicants must convey with

reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention." Vas-Cath Inc., 935 F.2d at 1563-64 (Fed. Cir. 1991).

Whether the written description requirements is satisfied is a fact-based inquiry that will depend on the nature of the claimed invention, Enzo, 23 F.3d at 963, and the knowledge of the one skilled in the art at the time an invention is made and a patent application is filed.

The Guidelines for Examination of Patent Application under 35 U.S.C Section 12, first paragraph "Written Description" requirement, 66 Fed. Reg. 1099 (Jan. 5, 2001) (Guidelines), an accurate description of the law for examining patent applications, and a persuasive authority, provide further guidance for determining whether the written description requirement is met for claims drawn to a genus. The Guidelines state:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species ... by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus.
>
> A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.
>
> * * *
>
> Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For invention in an unpredictable art, adequate written description of a genus

> which embraces widely variant species cannot be achieved by disclosing only one species within the genus.

Guidelines, 66 Fed. Reg. at 1106.

The Office Actions and the Advisory Action highlighted much of the foregoing general requirements for written description. However, the instant patent application is akin to the patent application in Capon v. Eshha, 418 F.3d 1349, 1358 (Fed. Cir. 2005).

In Capon, the Federal Circuit has elaborated on the written description requirement under first paragraph of 35. U.S.C. 112. In Capon, the Federal Circuit held that "what is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject mater." Id.

The invention in Capon involved "chimeric DNA that encodes single-chain chimeric proteins for expression on the surface of the cells of the immune system, plus expression vectors and cells transformed by the chimeric DNA. The Federal Circuit held that the Board of Patent Appeals and Interference erred in holding that the written description requirement was not met because the disclosure failed to "reiterate the structure or formula or chemical name of the nucleotide sequences of the claimed chimeric genes."

The Federal Circuit held in Capon that the prior art contained "extensive knowledge of the nucleotide structure of the various immune-related segments of DNA," including "over 785 mouse antibody DNA light chains and 1,327 mouse antibody DNA heavy chains." Id. at 1355.

Similarly to the patent application in Capon, the claim element "nucleoside antimetabolite" of the present application was well known to the one skilled in the art of preparing oligonucleotides with modified nucleosides at the time this application was filed.

Nucleoside is defined as a molecule a nitrogenous base and a pentose sugar (Lehninger – Principles of Biochemistry). The online encyclopedia (Wikipedia) provides the following description for nucleoside antimetabolite.

> An **antimetabolite** is a chemical that inhibits the use of a metabolite, which is another chemical that is part of normal metabolism. Such substances are often similar in structure to the metabolite that they interfere with.

> Antimetabolites can be used in cancer treatment,[3] as they interfere with DNA production and therefore cell division and the growth of tumors. Because cancer cells spend more time dividing than other cells, inhibiting cell division harms tumor cells more than other cells.
> Anti-metabolites masquerade as a purine (azathioprine, mercaptopurine) or a pyrimidine - which become the building blocks of DNA. They prevent these substances from becoming incorporated in to DNA during the S phase (of the cell cycle), stopping normal development and division.

The Paragraph 0076 of the instant patent application also provides the description for "nucleoside antimetabolite." Moreover, the Appendix entitled "**Scientific Publications describing the use of nucleoside antimetabolite prior to the filing of the Patent Application No. 10/768,996"** attached to this " Remarks" section also lists 36 peer-reviewed scientific publications discussing various antimetabolite nucleosides known in the art prior to filing of the instant patent application. A variety of antimetabolite nucleosides were known in the art prior to the filing of the instant application.

Therefore, the generic claim, claim 51 of this invention, does not depend on the use of any particular antimetabolite nucleosides *per se* in the treatment of cancer.

Rather, claim 51 embodies what the inventors have invented: a new genus of composition comprising an oligonucleotide with at least two CpG moieties and a nucleoside antimetabolite covalently linked to the oligonucleotide.

Claim 74 limits claim 51 to a special structural motif of this composition, and claim 89 discloses the method of making the nucleotide of claim 51.

Thus as per the standard for written description set forth by Federal Circuit in Capon, one of skill in the art would recognize that the applicants were in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed for an oligonucleotide with at lease two CpG moieties and a nucleotide antimetabolite covalently linked to the oligonucleotide.

For all the reasons presented above, it is respectfully submitted that the claims at issue are patentable. and that rejection with respect to claims 51, 54-74 and 76-90 should be withdrawn.

CONCLUSION

The Applicants have shown that the generic claims fulfill the written description requirements and that the applicants were in possession of the invention in accordance with the published guidelines and the guidance provided by the Examiner.

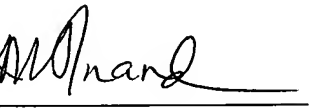
Furthermore, the Applicants invented a completely novel approach for fighting cancer and disclosed compelling experimental data. They have also disclosed how this technology may be replicated to target various forms of cancer by utilizing a variety of antimetabolites with cancer-fighting properties.

A Notice of Allowance for the claims presented, including the generic claims, is respectfully requested. Kindly contact the undersigned representative for any matter still outstanding in the case to put it into a condition for allowance.

Dated: January 9, 2010

Respectfully submitted,

Suresh C. Srivastava, Satya P. Bajpai and Kwok-Hung Sit
Applicants

By: 

Indu M. Anand
Registration Number: 52,557
(978) 250-9003/ (617) 930-5000

Scientific Publications describing the use of nucleoside antimetabolite prior to the filing of the Patent Application No. 10/768,996

This list is submitted in support of applicants' demonstration that the Written Description requirement is fulfilled. In particular, it gives evidence of the use of the term "Nucleoside Antimetabolite" as a term of art, and as it relates to the Written Description requirement.

This list is presented in two parts: A) Summary of each article cited; and (B) Abstracts of the articles.

(A) Summaries Of Articles Cited

1.Gemcitabine and Other Nucleoside Antimeabolites in Combination Chemotherapy;
The interaction of gemcitabine and cytarabine on murine leukemias L1210 or P388 and on human normal and leukemic cell growth in vitro; **Haematologica**, 2000, Vol 85, Issue 6, 588-594; E Lech-Maranda, A Korycka, and T Robak

Summary: Gemcitabine (dFdC) which is a new **nucleoside antimetabolite** of deoxycytidine that resembles cytarabine (Ara-C) in both its structure and metabolism and is also **a** nucleoside antimetabolite, were used in combination chemotherapy in leukemic cell growth.

2. Mode Of Action Of Gemcitabine; Gemcitabine (2',2'-Difluoro-2'-Deoxycytidine), an Antimetabolite That Poisons Topoisomerase;
Clinical Cancer Research August 2002 *8;* 2499; Philippe Pourquier1, Christopher Gioffre, Glenda Kohlhagen,Yoshimasa Urasaki2, François Goldwasser, Lary W. Hertel, Shuyuan Yu, Richard T. Pon, William H. Gmeiner and Yves Pommier

Summary: It is known that Gemcitabine gets incorporated into DNA, and results in concomitant inhibition of DNA synthesis. The article presents data on the mechanism of action of gemcitabine , a nucleoside antimetabolte and details of mode of incorporation.

3. Inhibitory effects of the nucleoside analogue gemcitabine on prostatic carcinoma cells; **Prostate,** March 1, 1996; 28(3): 172-81; MV Cronauer, H Klocker, H Talasz, FH Geisen, A Hobisch, C Radmayr, G Bock, Z Culig, M Schirmer, A Reissigl, G Bartsch, and G Konwalinka;

Summary: Gemcitabine (2',2'difluoro-2'deoxycytidine, dFdC) , a synthetic **nucleoside antimetabolite** a pyrimidine nucleotide. This article presents in vitro data showing strong effect on the proliferation and colony formation of the human androgen-sensitive tumor cell line LNCaP.

4. Preclinical and clinical studies with combinations of pemetrexed and gemcitabine. **Semin Oncol,** December 1, 2002; 29(6 Suppl 18): 30-4; AA Adjei

Summary: Gemcitabine which is a pyrimidine **nucleoside antimetabolite,** approved worldwide was used in combination chemoptherapy with another **antimetabolite** pemetrexed (Taxol; a non nucleoside antometabolite) which inhibits folate-dependent enzymes thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase.
The combination of two was used for phase I study and showed striking activity. Phase II studies of this combination were also started for breast and non-small cell lung cancer. According to this article, presumably due to success of phase 1 and phase II, in addition, a phase III study in pancreatic cancer is ongoing.

It is interesting to note that although Gemcitabine is quite active alone as drug, in order to be effective in variety of cancers, combination with another drug is preferably required for effective chemotherapy.

5. Gemcitabine and pemetrexed disodium combinations in vitro and in vivo.
Lung Cancer, December 1, 2001; 34 Suppl 4: S103-5; AA Adjei

Summary: This is another study similar to article # 4 above, which reports on combination chemotherapy of Pemetrexed disodium (ALIMTA; a non nucleoside **antimetabolite)** and gemcitabine. Preclinical studies showed cytotoxic synergy between gemcitabine and pemetrexed. Clinical activity with this combination was carried out in phase I and phase II for non-small cell lung cancer. According to the article an international phase II study of this combination in non-small cell lung cancer is ongoing.

6. Elaidic Acid – Ester of cytarabine (P-4055), a **nucleoside antimetabolite;**
Antitumor Activity of P-4055 (Elaidic Acid-Cytarabine) compared to Cytarabine in metastatic and s.c. human tumor Xenograft Models: Biological activity in melanoma cells was found to be highly superior to that of cytarabine; **Cancer Research** 59, 2944-2949, June 1, 1999; Knut Breistøl[1], Jan Balzarini, Marit Liland Sandvold, Finn Myhren, Marita Martinsen, Erik De Clercq and Øystein Fodstad

Summary: In this study cytarabine, a **nucleoside antimetabolite** was chemically modified to incorporate a long chain fatty acid ester in order to enhance better uptake in the cellular environment for treatment of human cancer. The **modified nucleoside antimetabolite** showed significant biological activity.

7. Gemcitabine in the treatment of ovarian cancer; Int. J. Gynecol. Cancer, January 1, 2001; 11 Suppl 1: 39-41; SW Hansen

Summary: In this study Gemcitabine, a **nucleoside antimetabolite** was studied as single anticancer agent. Additionally it was used in combination chemotherapy with one and two additional anticancer agents, gemcitabine-cisplatin or gemcitabine-paclitaxel, as well as combination of three drugs, gemcitabine, paclitaxel, and cisplatin or carboplatin to achieve greater efficacy. Thus not only combination of two but three drugs has been an approach for most effective cancer treatment.

8. Gemcitabine in the treatment of ovarian cancer; Ann. Onc., January 1, 1999; 10 Suppl 1: 51-3; SW Hansen, MK Tuxen, and C Sessa.

Summary: This study similar to the study as mentioned in previous article # 7 above, gemcitabine a new **nucleoside antimetabolite** gave poor effectiveness for ovarian cancer, but in combination with cisplatin or gemcitabine-paclitaxel induced much higher effectiveness. The study shows that the in order to increase effectiveness, combination
with other drugs is generally required.

9. Lack of in vivo cross resistance with gemcitabine against drug-resistant murine P388 leukemias; **Cancer Chemother. Pharmacol.,** January 1, 1996; 38(2): 178-80; W.R. Waud, KS Gilbert, G.B. Grindey, and J.F. Worzalla

Summary: This is an important study from Eli Lilly, the company which markets gemcitabine, a novel pyrimidine **nucleoside antimetabolite.** Cell lines which are resistance to various drugs when treated with gemcitabine exhibited no resistant to gemcitabine. The authors developed cell lines to study this a for the purpose of using gemcitabine alone or in combination chermotherapy to avoid the problems of cross ressistance to various anticancer drugs. The authors concluded to use gemcitabine in combination with 1-beta-D-arabinofuranosylcytosine, another **nucleoside antibiotics** to avoid cross resistance seen with gemcitabine alone and develop therapeutic synergism.

10. **Phase II trial of gemcitabine in advanced sarcomas ;Cancer,** June 15, 2002; 94(12): 3225-9; S. Okuno, J. Edmonson, M. Mahoney, J.C. Buckner, S. Frytak, and E. Galanis.

Summary: In this study, gemcitabine, a **nucleoside antimetabolite**, an analog of deoxycytidine active in several types of tumors was studied in sarcoma in humans. Some degree of toxicity was observed and the drug did not show promise to continue further studies.

11: Gemcitabine: a pharmacologic and clinical overview; Cancer Nurs., April 1, 1999; 22(2): 176-83; M. Barton-Burke.

Summary: The article reviews pharmacological and clinical therapy data for gemcitabine (GemzarR), a **nucleoside antimetabolite,** including its toxicity. The drug was approved by FDA in 1996 for treatment of several types of cancers.

12. Gemcitabine and carboplatin for patients with advanced non-small cell lung cancer. **Semin Oncol,** June 1, 2001; 28(3 Suppl 10): 4-9; Domine, V. Casado, L.G. Estevez, A. Leon, J.I. Martin, M. Castillo, G. Rubio, and F. Lobo.

Summary: In this study gemcitabine was used in combination chemotherapy with cisplatin to increase efficacy in cancer treament. Although combination chemotherapy has been acceptable mode of cancer treatment, the best way of practice of combination chemotherapy to minimize toxicity & cross resistance remains to be clearly defined.

13. Preliminary evaluation of influence of gemcitabine (Gemzar) on proliferation and neuroendocrine activity of human TT cell line: immunocytochemical investigations.**Folia Histochem. Cytobiol.,** January 1, 2001; 39(2): 187-8; J. Dadan, S. Wolczynski, B. Sawicki, L. Chyczewski, A. Azzadin, J. Dzieciol, and Z. Puchalski.

Summary: In this study gemcitabine, an antimetabolite nucleoside, was evaluated for treatment of medullary thyroid carcinoma (MTC) is total thyroidectomy. It is difficult to evaluate effectiveness of chemotherapy due to the rare incidence of MTC, and it was shown to have inhibitory influence on proliferative activity of TT cells used in the study.

A concentration-dependent inhibitory influence of gemcitabine on proliferative activity of TT cells was observed. It was also shown that the immunostaining was reduced,

14. The Antiproliferative Activity of DMDC Is Modulated by Inhibition of Cytidine Deaminase; **Cancer Research** 58, 1165-1169, March 15, 1998; Hiroyuki Eda, Masako Ura, Kaori F.-Ouchi, Yutaka Tanaka, Masanori Miwa and Hideo Ishitsuka

Summary: A new 2'-deoxycytidine (2'-dCyd) analogue, 2'-deoxy-2'-methylidenecytidine (DMDC), **a nucleoside antimetabolite** was found very promising as anticancer agent in multiple cancer cell lines. Study was carried to establish mode of actions and mechanism of action. Further combination chemotherapy of gemcitabine with another modified nucleoside tetrahydrouridine was evaluated and found to be encouraging.

15. **Nucleoside analogues in the treatment of haematological malignancies; Expert Opin. Pharmacother.,** June 1, 2001; 2(6): 929-43; S.A. Johnson.

Summary: This article reviews various nucleoside antimetabolites as cytotoxics. Many examples such as Cytrabine, Cladribine, fludarbine, gemcitabine. nelarabine, clofarabine and troxacitabine were choosen for detailed therapeutic properties/ index.
It is interesting to note that many of the anticancer agents are immunosuppressive in nature.

16. Synthesis of 1-(2-deoxy-2-isocyano-beta-D-arabinofuranosyl)cytosine and related nucleosides as potential antitumor agents; **J.Med Chem,** December 24, 1993; 36(26): 4190-4; A. Matsuda, A. Dan, N. Minakawa, S.J. Tregear, S. Okazaki, Y. Sugimoto and T. Sasaki; Nucleosides and nucleotides. 123.

Summary: This publication details synthesis of a new chemical modification of several nucleoside related to **nucleoside antimetabolite** beta-D-artabinofuranosycytosine, **nucleoside antimetabolite** beta-D-artabinofuranosyuracil and **nucleoside antimetabolite** beta-D-artabinofuranosythymine. Only moderate antitumor activity was observed.

17. Nucleosides as Antimetabolites: Thioguanine, mercaptopurine: their analogs and nucleosides as antimetabolites.**Curr Pharm Des,** January 1, 2003; 9(31): 2627-42; G.H. Elgemeie

Summary: This article presents an overview of well known purine based antimetabolites and various modifications of thiopurine based **nucleoside antimwetabolites.** In light of many toxic side effects of the thiopurine based **nucleoside antimetabolites** other approaches and modifications are discussed as safe therapeutic agents.

18. Metabolism of pyrimidine analogues and their nucleosides; **Pharmacol. Ther.,** January 1, 1990; 48(2): 189-222; G.C. Daher, B.E. Harris, and R.B. Diasio

Summary: The article discusses the mode of action of nucleoside antimetabolites and specifically pyrimidine nucleoside antimetabolites and how they cause cytotoxic effect within the cellular environment. Four most common pyrimidine based nucleoside antimetabolites, viz., fluorouracil, fluorodeoxyuridine, cytosine arabinoside and azacytidine.

19. Transport of Nucleoside antimetabolites in Cancer Cells; Nucleoside anticancer drugs: the role of nucleoside transporters in resistance to cancer chemotherapy; **Oncogene,** October 20, 2003; 22(47): 7524-36; V.L. Damaraju, S. Damaraju, J.D. Young, S.A. Baldwin, J. Mackey, M.B. Sawyer and C.E. Cass
antimetabolite into cells and outlines various possible mechanisms

Summary: The article discusses mechanism of transport of **nucleoside antimetabolite** into cells and outlines various factors such as as hENTs, hCNTs and their role in transport of cytotoxic chemotherapeutic **nucleoside** drugs. This understanding is very important towards the design of better **nucleoside antimetabolites.**

20. Potential Multifunctional Antitumor Nucleosides and Analogues; 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)-cytosine, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil, and their nucleobase analogues as new potential multifunctional antitumor nucleosides with a broad spectrum of activity; **J. Med. Chem.,** December 6, 1996; 39(25): 5005-11; H. Hattori, M. Tanaka, M.

Fukushima, T. Sasaki, and A. Matsuda; Nucleosides and nucleotides. 158.

Summary: This article describes synthesis of new modifications (1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil; EUrd) as a approach to develop multifunctional antitumor **nucleoside antimetabolite**. The authors introduced a "biochemically reactive" ethynyl group on uracil nucleoside resulting in modified uridine (beta-D-ribo-pentofuranosyl)uracil). However only moderate biological activity was observed.

21. **Antitumor activity** and pharmacokinetics of TAS-106, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine, **Jpn. J. Cancer Res,** March 1, 2001; 92(3): 343-51, Y. Shimamoto, A. Fujioka, H. Kazuno, Y. Murakami, H. Ohshimo, T. Kato, A. Matsuda, T. Sasaki, and M. Fukushima.

Summary: This article similar to the preceeding article describe synthesis of 3-C –ethynyl modification of cytosine nucleoside. This modification results in a modified nucleoside antimetabolite and possesses antitumor activity and strong cytotoxic effects useful for a cancer chemotherapy, and showed promise with lower less side effects.

22. Combinations of 5-fluorouracil and N-(2-Chloroethyl)-N-nitrosourea moieties separated by a three-carbon chain; The synthesis of antitumor activity in mice of molecular combinations of 5-fluorouracil and N-(2-Chloroethyl)-N-nitrosourea moieties separated by a three-carbon chain; **J. Med. Chem.,** March 29, 1996; 39(7): 1403-12; R.S. McElhinney, J.E. McCormick, M.C. Bibby, J.A. Double, M. Radacic, and P. Dumont; **Nucleoside analogs. 14.**

Summary: This article describes synthesis of modified nucleoside derived by combination of **nucleoside antimetabolite**, 5- fluoro uracil and attachmernt of N-(2-Chloroethyl)-N-nitrosourea moieties. Some antitumor activity was observed.

23. Modulation of the equilibrative nucleoside transporter by inhibitors of DNA synthesis; Br. J. Cancer, October 1, 1995; 72(4): 939-42; J. Pressacco, J.S. Wiley, G.P. Jamieson, C. Erlichman, and D.W. Hedley

Summary: This study was carried out to measure and modulate the activity of sensitive **nucleoside** transporter (es), at the stage of de novo nucleoside synthesis pathway and thereby to regulate **nucleoside antimetabolite**. Inhibitors of DNA synthesis such as hydroxyurea and 5-fluorouracil (5-FU), which inhibit the de novo synthesis of DNA precursors, produced increases in the expression of es, while cytosine arabinoside (ara-C), another **nucleoside antimetabolite** produced no significant increase in es expression.

24. In **Vitro Cell Dev Biol; Br. J. Cancer,** October 1, 1995; 72(4): 939-42.

November 1, 1991; 27A (11): 873-7; M. Moorghen, P. Ince, K.J. Finney, A.J. Watson, and A.L. Harris, Department of Pathology, University of Newcastle upon Tyne, United Kingdom

Summary: Nucleoside transport inhibitors modulate biological activity of **nucleoside antimetabolites.** The effect of nucleoside transport inhibitors such as nitrobenzylthioinosine (NBMPR) and dipyridamole which are responsible for binding with the enzymes responsible for transport of nucleosides was studied in this article.

25. Potentiation of the cytotoxicity of thymidylate synthase (TS) inhibitors by dipyridamole analogues with reduced alpha1-acid glycoprotein binding.
Br. J. Cancer, August 1, 1999; 80(11): 1738-46; N.J. Curtin, K.J .Bowman, R.N. Turner, B. Huang, P.J. Loughlin, A.H. Calvert, B.T. Golding, R.J. Griffin and D.R. Newell.

Summary: A new approach has been used by the authors to enhance the biological activity of the **nucleoside antimetabolites** by developing nucleoside transport inhibitors. A number of dipyridamole were shown to have potency of inhibition of uptake of nucleosides, there by inhibition of DNA synthesis.

26. Characterization of a multidrug resistant human erythroleukemia cell line (K562) exhibiting spontaneous resistance to 1-beta-D-arabinofuranosylcytosine; **Leukemia,**
S. Grant, A. Turner, P. Nelms and S. Yanovich; May 1, 1995; 9(5): 808-14.

Summary: One of the key problems associated with anticancer drugs is multi drug resistance (MDR) during chemotherapy. In this article the authors studied the mechanism of MDR in the case of **nucleoside analog antimetabolite** 1-beta-D-arabinofuranosylcytosine (ara-C). Formation of the monophosphate of these nucleosides and the enzymes responsible for the phosphorylation seems to be the factor controlling resistance.

27. Clofarabine: Bioenvision/ILEX; Curr Opin Investig Drugs,
A. Sternberg; December 1, 2003; 4(12): 1479-87;

Summary: The article discusses a new modified **nucleoside antimetabolite,** Clofarabine which has shown significant promise for treatment of various forms of tumors and various forms of cancers.

28. Corticosteroid responsive fludarabine pulmonary toxicity, **Am. J. Clin. Oncol.,** August 1, 2002; 25(4): 340-1, G.S. Stoica, H.E. Greenberg and L.J. Rossoff.
Division of Pulmonary and Critical Care Medicine, Long Island Jewish Medical Center, New Hyde Park, New York 11042-1101, U.S.A

Summary: Not only modified nucleosides (with a free 5'- hydroxyl group) are **nucleoside antimetabolites**, but the corresponding 5'-mono phosphates of these nucleosides are also **nucleoside antimetabolites** and work with the same principle as the free 5'- hydroxyl nucleosides, i.e., getting incorporated during DNA synthesis and eventually stopping the DNA synthesis. Fludarabine, which has a fluoro group at 2- position was developed by introducing fluorine into a known **nucleoside antimetabolite**; Ara-A (9-beta-D-arabinofuranosyl adenine; vidarabine). The article
reports clinical efficacy data on this nucleoside antimetabolite and the contribution to toxicity.

29. Cerebrospinal fluid pharmacokinetics and **toxicology of intraventricular and intrathecal arabinosyl-5-azacytosine** (fazarabine, NSC 281272) in the nonhuman primate.**Invest New Drugs,** May 1, 1993; 11(2-3): 135-40, R.L. Heideman, C. McCully, F.M. Balis and D.G. Poplack.

Summary: 5- aza-2'- deoxy cytidine and 5- aza- cytidine are highly potent anticancer drugs and presently used in cancer chemotherapy. Arabinosyl-5-azacytosine (AAC), a
new **nucleoside antimetabolite**, is similar to 5- aza-2'- deoxy cytidine and 5- aza- cytidine in structure and has also shown strong anti tumor activity. The present article report clinical evaluation on primates.

30. Phase I trial and biochemical evaluation of tiazofurin administered on a weekly schedule, **Sel. Cancer Ther.,** March 1, 1990; 6(1): 51-61, T.J. Melink, G. Sarosy, A.R. Hanauske, J.L. Phillips, J.H. Bayne, M.R. Grever, H.N. Jayaram and D.D. Von Hoff.

Summary: The article reports pharmacological and biochemical study with another nucleoside antimetabolite, tiazofurin (2-B-D-Ribofuranosylthiazole-4-Carboxamide: NSC 286193). These are another class of nucleoside antimetabolites which act on the biosynthesis pathway of synthesis of purine nucleosides themselves. This leads to the inhibition of DNA synthesis and antitumor properties. However this compound was found to be associated with significant level of cellular toxicities.

31. Evaluation of purine and pyrimidine analogues in human tumor cells from patients with low-grade lymphoproliferative disorders using the FMCA, **Eur. J. Haematol,** May 1, 1999; 62(5): 293-9, Aleskog, R Larsson, M Hoglund, C Sundstrom, and J Kristensen.

Summary: This article reports clinical study data with few well established **pyrimidine antimetabolites**, fludarabine, cladribine (CdA), cytarabine (AraC) and gemcitabine.
Cytotoxic studies carried out revealed effectiveness against non – Hodgkins lymphoma (NHL) is active against low-grade NHL and against acute leukemia. Gemcitabine and AraC were shown to be promising against low-grade NHL.

32. Altered susceptibility of differentiating HL-60 cells to apoptosis induced by antitumor drugs, Leukemia, February 1, 1994; 8(2): 281-8, G Del Bino, X. Li, F. Traganos, and Z. Darzynkiewicz.

Summary: In this study it was shown that effectiveness of chemotherapeutic agents, **nucleoside antimetabolite,** including radiation is most likely reduced if a drug or chemical which has capability of cell cycle differentiation such as during S- phase or apoptosis and is administered first. In the converse, cell death or enhancement of apoptosis is expected if the cell cycle differentiating drug or chemical is administered in the reverse sequence.

33. Polarographic properties and **potential carcinogenicity of some natural nucleosides and their synthetic analogues; Bioelectrochem Bioenerg,** February 1, 1999; 48(1): 129-34.; L. Novotny, A.Vachalkova, and A Piskala

Summary: A series of natural, synthetic nucleosides selected from a group of **Nucleoside antimetabolites** were studied for their potential carcinogenicity. It is interesting to note that various **nucleoside antimetabolites** do possess carcinogenicity.

34. This articles were taken from the book" **Drug Resistance and Selectivity, Biochemical and Cellular Basis , Edited by Enrico Mihich, Roswell Park Memorial Cancer Institute, Buffalo, NY,; Academic Press, 1973, Pages 83-93,** Chapter 3. CROSS-RESISTANCE AND COLLATERAL SENSITIVITY; Dorris J. Hutchinson and Franz A. Schmid"

Summary: The articles covering many **purine and pyrimidine antimetabolites** analogs are part of the chapter of this book Edited by E. Michich of Roswell Park Memorial Insititute, Buffalo, NY, and were written by many well known scientists involved in anticancer chemotherapy field in general. The cross resistance to anti cancer drugs and specifically **nucleoside antimetabolite** of modified purine and pyrimidine had been a serious issue and challenge recognized early on. Various mechanisms of this phenomenon were studied While nucleoside antimetabolites are very promising and effective in cancer chemotherapy, the issues addressed in the chapter have been serious and responsible for ineffectiveness and as well as associated significant cellular toxicity in general. The articles presents various approaches to address and overcome the shortcomings

Scientific Publications describing the use of antimetabolite nucleoside prior to the filing of the Patent Application No. 10/768,996

(B) Abstracts Of The Articles

1.Gemcitabine and Other Nucleoside Antimeabolites in Combination Chemotherapy;

The interaction of gemcitabine and cytarabine on murine leukemias L1210 or P388 and on human normal and leukemic cell growth in vitro; **Haematologica**, 2000, Vol 85, Issue 6, 588-594; E Lech-Maranda, A Korycka, and T Robak

BACKGROUND AND OBJECTIVE: Gemcitabine (dFdC) is a new **nucleoside antimetabolite** of deoxycytidine that resembles cytarabine (Ara-C) in both its structure and metabolism. Little is known about dFdC efficacy in hematologic malignancies, either as a single drug or in combination with other drugs. In this study we have tried to determine whether the cytotoxic effect of Ara-C can be increased by using it in combined therapy with dFdC. DESIGN AND METHODS: In the in vivo part of our study, mice bearing L1210 or P388 leukemia were treated with dFdC and Ara-C. The drugs were administered alone and in combination according to the following schedules: Ara-C and dFdC at the same time, dFdC before Ara-C, and Ara-C before dFdC. The efficacy of the therapy against leukemia (defined as the increase in lifespan, ILS) was assessed as the percentage of the median survival time (MST) of the treated group (T) in relationship to that of the control group (C): ILS=[(MST(C)/MST(T)) -1]x100. In the in vitro part of our study, normal granulocyte-macrophage colony-forming unit (CFU-GM) cells as well as CFU-GM cells obtained from patients with chronic myeloid leukemia (CML) were incubated either with dFdC or Ara-C alone or with adequate concentrations of a combination of these drugs. RESULTS: The in vivo experiment revealed that in both leukemias tested, combined therapy with dFdC given before Ara-C and dFdC given at the same time with Ara-C were more effective than monotherapy with either dFdC or Ara-C. The other treatment schedule (Ara-C before dFdC) did not significantly prolong the survival time of the treated mice bearing L1210 or P388 leukemia as compared with the treatment with dFdC alone. The in vitro experiments showed that dFdC used together with Ara-C acted additively on normal as well as CML CFU-GM cells. Furthermore, the drugs used jointly inhibited the growth of colonies formed by CML CFU-GM cells to a significantly higher degree than normal CFU-GM and the differences were statistically significant in the case of the combination of highest concentrations. INTERPRETATION AND CONCLUSIONS: Gemcitabine increased the activity of Ara-C. As these agents incorporate into DNA blocking chain elongation, and moreover, dFdC influences the cytotoxicity of Ara-C, our results could be explained by the drugs acting at these levels. dFdC used jointly with Ara-C may have an important clinical implication in the treatment of CML and other hematologic malignancies in future.

2. Mode Of Action Of Gemcitabine Gemcitabine (2′,2′-Difluoro-2′-Deoxycytidine), an Antimetabolite That Poisons Topoisomerase;
Clinical Cancer Research August 2002 *8;* 2499; Philippe Pourquier1, Christopher Gioffre, Glenda Kohlhagen,Yoshimasa Urasaki2, François Goldwasser, Lary W. Hertel, Shuyuan Yu,Richard T. Pon, William H. Gmeiner and Yves Pommier

Gemcitabine-containing regimens are among standard therapies for the treatment of advanced non-small cell lung,pancreatic, or bladder cancers. Gemcitabine is a nucleoside analogue and its cytotoxicity is correlated with incorporation into genomic DNA and concomitant inhibition of DNA

synthesis. However, it is still unclear by which mechanism(s) gemcitabine incorporation leads to cell death.
Experimental Design: We used purified oligodeoxynucleotides to study the effects of gemcitabine incorporation on topoisomerase I (top1) activity and tested the role of top1 poisoning in gemcitabine-induced cytotoxicity in cancer cells.
Results: We found that top1-mediated DNA cleavage was enhanced when gemcitabine was incorporated immediately 3′ from a top1 cleavage site on the nonscissile strand. This position-specific enhancement was attributable to an increased DNA cleavage by top1 and was likely to have resulted from a combination of gemcitabine-induced conformational and electrostatic effects. Gemcitabine also enhanced camptothecin-induced cleavage complexes. We also detected top1 cleavage complexes in human leukemia CEM cells treated with gemcitabine and a 5-fold resistance of P388/CPT45 top1-deficient cells to gemcitabine, indicating that poisoning of top1 can contribute to the antitumor activity of gemcitabine.
Conclusions: The present results extend our recent finding that incorporation of 1-β-D-arabinofuranosylcytosine into DNA can induce top1 cleavage complexes [P. Pourquier *et al.* Proc. Natl. Acad. Sci. USA, *97:* 1885–1890, 2000]. The enhancement of camptothecin-induced top1 cleavage complexes may, at least in part, contribute to the synergistic or additive effects of gemcitabine in combination with topotecan and irinotecan in human breast or lung cancer cells.

3. Inhibitory effects of the nucleoside analogue gemcitabine on prostatic carcinoma cells; **Prostate,** March 1, 1996; 28(3): 172-81; MV Cronauer, H Klocker, H Talasz, FH Geisen, A Hobisch, C Radmayr, G Bock, Z Culig, M Schirmer, A Reissigl, G Bartsch, and G Konwalinka; Department of Urology, University of Innsbruck, Austria

Gemcitabine (2',2'difluoro-2'deoxycytidine, dFdC) is a synthetic **antimetabolite** of the cellular pyrimidine nucleotide metabolism. In a first series of in vitro experiments, the drug showed a strong effect on the proliferation and colony formation of the human androgen-sensitive tumor cell line LNCaP and the androgen-insensitive cell lines PC-3 and DU-145. Maximal inhibition occurred at a dFdC concentration as low as 30 nM. In contrast to the cell lines which were derived from metastatic lesions of prostate cancer patients, no inhibitory effects were found in normal primary prostatic epithelial cells at concentrations up to 100 nM. The effect of gemcitabine was reversed by co-administration of 10-100 microM of its natural analogue deoxycytidine. In view of a future clinical application of this anti-tumor drug in advanced prostatic carcinoma, we have compared the effect of gemcitabine on prostatic tumor cells with that on bone marrow granulopoietic-macrophage progenitor cells, because neutropenia is a common side effect of gemcitabine treatment. The time course of action on the two kinds of cells was markedly different. Colony formation of tumor cells was inhibited by two thirds at a gemcitabine concentration of about 3.5 nM. The same effect on granulopoietic-macrophagic progenitor cells required a concentration of 9 nM. Co-administration of deoxycytidine to gemcitabine-treated tumor cell cultures completely antagonized the effect of gemcitabine whereas addition of deoxycytidine after 48 hr of gemcitabine treatment could not prevent gemcitabine action on the tumor cells. In contrast, more than half of the granulopoietic-

macrophagic progenitor cells could still be rescued by deoxycytidine administration after 48 hr. These findings and the marked difference in the susceptibility of neoplastic and normal prostatic cells suggest that gemcitabine is a promising substance which should be further evaluated as to its efficacy in the treatment of advanced prostatic carcinoma.

4. Preclinical and clinical studies with combinations of pemetrexed and gemcitabine.
Semin Oncol, December 1, 2002; 29(6 Suppl 18): 30-4; AA Adjei

The novel **antimetabolite** pemetrexed inhibits the folate-dependent enzymes thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase. This agent is broadly active in a wide variety of solid tumors, including non-small cell lung, breast, bladder, head and neck, and ovarian cancers, as well as mesothelioma. Gemcitabine is a pyrimidine **nucleoside antimetabolite** that is approved worldwide for the treatment of pancreatic and non-small cell lung cancers, and bladder cancer outside the United States. In addition, gemcitabine is active against a broad range of tumors including breast, ovarian, and other cancers. Preclinical studies have shown cytotoxic synergy when pemetrexed is combined with gemcitabine. Based on these data, a phase I study of this combination was performed that showed striking activity. Phase II studies of this combination are being performed in breast and non-small cell lung cancer. In addition, a phase III study in pancreatic cancer is ongoing.

5. Gemcitabine and pemetrexed disodium combinations in vitro and in vivo.
Lung Cancer, December 1, 2001; 34 Suppl 4: S103-5; AA Adjei

Pemetrexed disodium (ALIMTA) is a novel **antimetabolite** that inhibits at least three folate-dependent enzymes, thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase. Pemetrexed disodium is broadly active in a wide variety of solid tumours, including non-small cell lung, breast, bladder, head and neck and ovarian cancers. Gemcitabine is a broadly active pyrimidine **nucleoside antimetabolite**, which is approved for the treatment of pancreatic and non-small cell lung cancers. Three preclinical studies have been reported that show cytotoxic synergy between gemcitabine and pemetrexed. Clinical activity with this combination has been observed in a phase I study, with partial responses in three of five patients previously treated for non-small cell lung cancer. An international phase II study of this combination in non-small cell lung cancer is ongoing.

6. Elaidic Acid – Ester of cytarabine (P-4055), a nucleoside antimetabolite;
Antitumor Activity of P-4055 (Elaidic Acid-Cytarabine) Compared to Cytarabine in Metastatic and s.c. Human Tumor Xenograft Models: Biological activity in melanoma cells was found to be highly superior to that of cytarabine
Cancer Research 59, 2944-2949, June 1, 1999; Knut Breistøl[1], Jan Balzarini, Marit Liland Sandvold, Finn Myhren, Marita Martinsen, Erik De Clercq andØystein Fodstad

The antineoplastic efficacy of P-4055, a 5'-elaidic acid (C18:1, unsaturated fatty acid) ester of cytarabine, a **nucleoside antimetabolite** frequently used in the treatment of hematological malignancies, was examined in several *in vivo* models for human cancer.

In initial dose-finding studies in nude mice, the efficacy of P-4055 was highest when using schedules with repeated dailydoses. In a Raji Burkitt's lymphoma leptomeningeal carcinomatosis model in nude rats, the control cytarabine- and saline-treated animals (five in each group) had a mean survival time of 13.2 days, whereas treatment with P-4055 resulted in three of five long-time survivors (>70 days). In a systemic Raji leukemia model in nude mice, 8 of 10 of the P-4055-treated animals survived (>80 days), compared with none of the cytarabine-treated animals (mean survival time, 34.2 days).

In s.c. xenograft models, the effects of maximum tolerated doses of P-4055 and cytarabine, given in four weekly cycles of daily bolus i.v. injections for 5 subsequent days, against seven tumors (three melanomas, one lung adenocarcinoma, one breast cancer, and two osteogenic sarcomas) were investigated. P-4055 induced partial or complete tumor regression of the lung carcinoma, as well as of all three malignant melanomas. In two of the melanomas the activity was highly superior to that of cytarabine, and both P-4055 and cytarabine were, in general, more effective than several clinically established drugs previously tested in the same tumor models. In *in vitro* studies, inhibitors of **nucleoside** carrier-dependent transport, nitrobenzylmercaptopurine riboside and dipyridamol, reduced strongly the cellular sensitivity to cytarabine, but not to P-4055, indicating that P-4055 uses an alternative/additional mechanism of internalization into the cell compared with cytarabine. The results explain, at least in part, the observed differences between the two compounds in *in vivo* efficacy, and together the data strongly support the evaluation of P-4055 in clinical studies.

7. Gemcitabine in the treatment of ovarian cancer; Int. J. Gynecol. Cancer, January 1, 2001; 11 Suppl 1: 39-41; SW Hansen

Gemcitabine is a **nucleoside antimetabolite** with established activity against several solid tumors. The activity of the drug in patients with ovarian cancer has been reviewed both in patients who have received single drug treatment and in patients who have received combination chemotherapy. The response rates, with single agent gemcitabine, range from 13 to 24% both in previously treated and untreated patients. Doublets consisting of gemcitabine-cisplatin or gemcitabine-paclitaxel, in previously treated patients, induced response in 53% and 40% of the patients, respectively. In three studies, first-line treatment with the combination of cisplatin and gemcitabine induced remission in 53% to 71% of the patients. The triplet, including gemcitabine, paclitaxel, and cisplatin or carboplatin, has been examined in previously treated patients and a response rate of 100% was observed. In previously untreated patients the combination of gemcitabine, paclitaxel, and carboplatin has been preferred due to a more favorable toxicity profile. The activity of this combination, observed in 25 evaluable patients, was very high as all patients responded. Complete

remission was observed in 60% of the patients and partial remission in 40%. Based on these promising data the triplet consisting of gemcitabine, paclitaxel, and carboplatin has been included in randomized trials both in the US and in Europe.

8. Gemcitabine in the treatment of ovarian cancer; Ann. Onc., January 1, 1999; 10 Suppl 1: 51-3; SW Hansen, MK Tuxen, and C Sessa.

Gemcitabine is a new **nucleoside antimetabolite** with established activity against solid tumours. In previously treated patients the response rate with the drug alone was around 13%. Combination therapy with gemcitabine-cisplatin or gemcitabine-paclitaxel induced responses in 53 and 40% respectively. In previously untreated patients with poor prognostic features a 24% response rate was reported for the drug alone, but in combination with cisplatin remissions were found in 53%-71% of patients. Gemcitabine, paclitaxel, and carboplatin (or cisplatin) in combination appeared to be a feasible and active combination. In a pilot with eight previously treated patients all obtained a remission and in untreated patients a remission occurred in all evaluable patients either clinically or measured by a decrease of CA 125. Dose-limiting toxicity is mainly haematological.

9. Lack of in vivo crossresistance with gemcitabine against drug-resistant murine P388 leukemias; **Cancer Chemother. Pharmacol.,** January 1, 1996; 38(2): 178-80; W.R. Waud, KS Gilbert, G.B. Grindey, and J.F. Worzalla

Gemcitabine, a novel pyrimidine **nucleoside antimetabolite**, has shown clinical antitumor activity against several tumors (breast, small-cell and non-small-cell lung, bladder, pancreatic, and ovarian). We have developed a drug-resistance profile for gemcitabine using eight drug-resistant P388 leukemias in order to identify potentially useful guides for patient selection for further clinical trials of gemcitabine and possible non crossresistant drug combinations with gemcitabine. Multidrug-resistant P388 leukemias (leukemias resistant to doxorubicin or etoposide) exhibited no cross resistance to gemcitabine. Leukemias resistant to vincristine (not multidrug resistant), cyclophosphamide, melphalan, cisplatin, and methotrexate were also not cross resistant to gemcitabine. Only the leukemia resistant to 1-beta-D-arabinofuranosylcytosine was cross resistant to gemcitabine. The results suggest that (1) it may be important to exclude or to monitor with extra care patients who have previously been treated with 1-beta-D-arabinofuranosylcytosine and (2) the lack of cross resistance seen with gemcitabine may contribute to therapeutic synergism when gemcitabine is combined with other agents.

10. **Phase II trial of gemcitabine in advanced sarcomas ;Cancer,** June 15, 2002; 94(12): 3225-9; S. Okuno, J. Edmonson, M. Mahoney, J.C. Buckner, S. Frytak, and E. Galanis.

BACKGROUND: Care for patients with advanced sarcomas is mainly palliative. Gemcitabine, a**nucleoside antimetabolite**, is an analog of deoxycytidine that has shown antitumor activity in several tumors. The aim of the current study was to determine the clinical activity of gemcitabine in

patients with sarcomas. METHODS: The authors evaluated gemcitabine in patients with histologically confirmed sarcomas; one prior exposure to chemotherapy treatment was allowed. Prior radiation was allowed if given to non-indicator lesions. Treatment consisted of gemcitabine 1250 mg/m(2) intravenously over 30 minutes, every week x three, cycles repeated q28 days. RESULTS: Twenty nine of 30 patients were evaluable; one patient refused to initiate study treatment. The mean age was 50 years (range, 22-81 years); 59% were male, and 35% had an Eastern Cooperative Oncology Group performance status of 0 (vs. 1 or 2). Patients were histologically classified as leiomyosarcoma (seven gastrointestinal, four retroperitoneal, two inferior vena caval, three of the extremity, and two uterine), synovial (two patients), malignant fibrous histiocytoma (two patients), fibrosarcoma (one patient), osteosarcoma (two patients), liposarcoma (one patient), hemangiosarcoma (one patient), or giant cell (one patient). Patients received an average of two cycles (range, one to eight). Eighty three percent of patients discontinued treatment due to progression and 14% due to toxicity/refusal. Hematologic toxicities >or= Grade 3 were seen in 32% of patients and consisted of leukopenia and thrombocytopenia. Anorexia (Grade 1/2 in 6 patients, Grade 3 in 1 patient), nausea (Grade 1/2 in 7 patients, Grade 3 in 1 patient), and lethargy (Grade 1/2 in 19 patients) were the most frequently observed nonhematologic toxicities. One patient experienced Grade 3 edema and muscle infarction. A different patient experienced unexplained Grade 3 chest pain. One partial response was observed in a uterine leiomyosarcoma patient lasting at least three months. Overall response rate was 3% (95% confidence interval [CI]: 0-15). Median time -to progression was 2.1 months (95% CI: 1.8-3.0). CONCLUSIONS: The current gemcitabine regimen demonstrated acceptable levels of toxicity, but it failed to produce the number of responses needed to justify expansion of the current study. This regimen is not recommended for advanced sarcomas.

11: **Gemcitabine: a pharmacologic and clinical overview; Cancer Nurs.,** April 1, 1999; 22(2): 176-83; M. Barton-Burke.

There have been exciting new developments in anticancer therapy over the past few years. One such therapy uses gemcitabine (GemzarR), an **antimetabolite** approved in 1996 by the Food and Drug Administration (FDA) for first-line treatment of locally advanced (nonresectable stage II or stage III) or metastatic (stage IV) adenocarcinoma of the pancreas. This novel **nucleoside** analog resembles the naturally occurring pyrimidine **nucleoside** deoxycytidine, but it has a unique mechanism of action. Clinical studies with gemcitabine have demonstrated anticancer activity in pancreatic cancer; non-small-cell lung cancer; breast, bladder, and ovarian cancers; and small-cell lung cancer. Clinical trials in patients with cancer of the pancreas used a novel study end point called clinical benefits response (CBR) to measure gemcitabine's effect on disease-related symptoms. The CBR is a composite assessment of performance status, pain, and weight gain. Studies show that gemcitabine has a relatively mild safety profile, with myelosuppression as the major dose-limiting toxicity. The aim of this review is to provide the oncology nurse with an overview of gemcitabine's pharmacology, innovative clinical trial end points, and clinical performance, as well as the nursing care required for the patient receiving this drug.

12. Gemcitabine and carboplatin for patients with advanced non-small cell lung cancer. **Semin Oncol,** June 1, 2001; 28(3 Suppl 10): 4-9; Domine, V. Casado, L.G. Estevez, A. Leon, J.I. Martin, M. Castillo, G. Rubio, and F. Lobo.

The survival of patients with advanced non-small cell lung cancer remains poor. Cisplatin-based chemotherapy produces a modest benefit in survival compared with that observed with best supportive care. Gemcitabine (Gemzar; Eli Lilly and Company, Indianapolis, IN), a novel **nucleoside antimetabolite**, is active and well tolerated. The combination of gemcitabine/cisplatin has shown a significant improvement in response rate and survival over cisplatin alone. Phase III trials comparing gemcitabine/cisplatin with older combinations such as cisplatin/etoposide or mitomycin/ifosfamide/cisplatin have shown a higher activity for gemcitabine/cisplatin; however, the best way to combine these drugs remains unclear. In addition, the 3-week schedule has obtained a higher dose intensity with less toxicity and similar efficacy as the 4-week schedule. The role of carboplatin in combination with new drugs is still under evaluation. Gemcitabine/carboplatin seems to be a good alternative, with the advantage of ambulatory administration and lower nonhematologic toxicity. The 4-week schedule has produced frequent grade 3/4 neutropenia and thrombocytopenia in some studies. The 3-week schedule, using gemcitabine on days 1 and 8 and carboplatin on day 1, is a convenient and well-tolerated regimen. The toxicity profile is acceptable without serious symptoms. This schedule could be considered a good option as a standard regimen.

13. Preliminary evaluation of influence of gemcitabine (Gemzar) on proliferation and neuroendocrine activity of human TT cell line: immunocytochemical investigations.**Folia Histochem. Cytobiol.,** January 1, 2001; 39(2): 187-8; J. Dadan, S. Wolczynski, B. Sawicki, L. Chyczewski, A. Azzadin, J. Dzieciol, and Z. Puchalski.

The choice treatment of medullary thyroid carcinoma (MTC) is total thyroidectomy. It is difficult to evaluate effectiveness of chemotherapy due to the rare incidence of MTC. Gemcitabine is a new drug of **antimetabolite nucleoside** group used in treatment of cancers since 1996. The aim of this study was to evaluate the influence of gemcitabine on proliferation and neuroendocrine activity of human TT cell line derived from MTC. The cells were exposed to gemcitabine in the concentration of 10, 25 and 50 microg/ml for 24 hours. Immunocytochemical examinations were carried out by the method of avidin-biotin peroxidase complex (ABC) according to Hsu et al. to detect calcitonin, chromogranin A, synaptophysin and neuron-specific enolase in TT cells. A concentration-dependent inhibitory influence of gemcitabine on proliferative activity of TT cells was observed. It was also shown that the immunostaining was reduced, especially in case of neuron-specific enolase. Only the reaction detecting calcitonin was enhanced in persisting.
A concentration-dependent inhibitory influence of gemcitabine on proliferative activity of TT cells was observed. It was also shown that the immunostaining was reduced,has action mechanisms similar to those of DMDC, is only slightly active in tumors with higher levels of the enzyme. In the

present study, we investigated the roles of Cyd deaminase in the antitumor activity of the two 2'-dCyd **antimetabolites** in 13 human cancer cell lines. Tetrahydrouridine, an inhibitor of Cyd deaminase, reduced the antiproliferative activity of DMDC ($P = 0.0015$). Furthermore, tumor cells transfected with the gene of human Cyd deaminase become more susceptible to DMDC both *in vitro* and *in vivo*. These results indicate that Cyd deaminase is indeed essential for the activity of DMDC. In contrast, the antiproliferative activity of gemcitabine was increased to some extent by tetrahydrouridine ($P = 0.0277$), particularly in tumor cell lines with higher levels of Cyd deaminase. This suggests that higher levels of Cyd deaminase may inactivate gemcitabine. Among **nucleoside**s and deoxy**nucleoside**s tested, only dCyd, a natural substrate of both Cyd deaminase and dCyd kinase, suppressed the antiproliferative activity of DMDC by up to 150-fold. Because the $V_{max}K_m$ of DMDC for dCyd kinase was 8-fold lower than that for dCyd, the activation of DMDC to DMDC monophosphate (DMDCMP) by dCyd kinase might be competitively inhibited by dCyd. In addition, the dCyd concentrations in human cancer xenografts were inversely correlated with levels of Cyd deaminase activity. It is therefore suggested that higher levels of Cyd deaminase reduce the intrinsic cellular concentrations of dCyd in tumors, resulting in efficient activation of DMDC to DMDCMP by dCyd kinase. These results indicate that the efficacy of DMDC may be predicted by measuring the activity of Cyd deaminase in tumor tissues before treatment starts and that DMDC may be exploited in a new treatment modality: tumor enzyme-driven cancer chemotherapy.

14. The Antiproliferative Activity of DMDC Is Modulated by Inhibition of Cytidine Deaminase; **Cancer Research** 58, 1165-1169, March 15, 1998; Hiroyuki Eda, Masako Ura, Kaori F.-Ouchi, Yutaka Tanaka, Masanori Miwa and Hideo Ishitsuka

We showed that the efficacy of the new 2'-deoxycytidine (2'-dCyd) analogue **antimetabolite** 2'-deoxy-2'-methylidenecytidine (DMDC) correlates well with tumor levels of cytidine (Cyd) deaminase in human cancer xenograft models. DMDC was highly effective in tumors with higher levels of Cyd deaminase, whereas lower levels yielded only slight activity. In contrast, gemcitabine (2',2'-difluorodeoxycytidine), which has action mechanisms similar to those of DMDC, is only slightly active in tumors with higher levels of the enzyme. In the present study, we investigated the roles of Cyd deaminase in the antitumor activity of the two 2'-dCyd **antimetabolites** in 13 human cancer cell lines. Tetrahydrouridine, an inhibitor of Cyd deaminase, reduced the antiproliferative activity of DMDC ($P = 0.0015$). Furthermore, tumor cells transfected with the gene of human Cyd deaminase become more susceptible to DMDC both *in vitro* and *in vivo*. These results indicate that Cyd deaminase is indeed essential for the activity of DMDC. In contrast, the antiproliferative activity of gemcitabine was increased to some extent by tetrahydrouridine ($P = 0.0277$), particularly in tumor cell lines with higher levels of Cyd deaminase. This suggests that higher levels of Cyd deaminase may inactivate gemcitabine. Among **nucleoside**s and deoxy**nucleoside**s tested, only dCyd, a natural substrate of both Cyd deaminase and dCyd kinase, suppressed the antiproliferative activity of DMDC by up to 150-fold. Because the $V_{max}K_m$ of DMDC for dCyd kinase was 8-fold lower than that for dCyd, the activation

of DMDC to DMDC monophosphate (DMDCMP) by dCyd kinase might be competitively inhibited by dCyd. In addition, the dCyd concentrations in human cancer xenografts were inversely correlated with levels of Cyd deaminase activity. It is therefore suggested that higher levels of Cyd deaminase reduce the intrinsic cellular concentrations of dCyd in tumors, resulting in efficient activation of DMDC to DMDCMP by dCyd kinase. These results indicate that the efficacy of DMDC may be predicted by measuring the activity of Cyd deaminase in tumor tissues before treatment starts and that DMDC may be exploited in a new treatment modality: tumor enzyme-driven cancer chemotherapy.

15. **Nucleoside analogues in the treatment of haematological malignancies; Expert Opin. Pharmacother.,** June 1, 2001; 2(6): 929-43; S.A. Johnson.

The **nucleoside** analogues are a group of **antimetabolite** cytotoxics which generally have to be metabolized to the equivalent nucleotide before incorporation into DNA. Cytarabine is a well established component of the treatment of acute leukaemias and has its principal action on dividing cells. New formulations include a liposome encapsulated product for intrathecal use and oral cytarabine ocfosfate which may be suitable for long-term outpatient use. Pentostatin acts by causing accumulation of deoxynucleotides and, although active against hairy cell leukaemia, is associated with a poor tolerance profile. Cladribine and fludarabine have substantial activity in the treatment of chronic lymphocytic leukaemia (CLL) and low-grade non-Hodgkin's lymphoma (NHL). Fludarabine is the more thoroughly investigated of the two and is currently being developed in combination therapies for CLL and NHL and also in a combination with cytarabine for acute myeloid leukaemia. Fludarabine's immunosuppressive activity is being exploited in the conditioning of patients for non-myeloablative stem cell transplantation. Gemcitabine is an established agent in the treatment of a number of solid tumours but also has activity in haematological malignancies which might be exploited by the use of extended infusion schedules. Newer agents including nelarabine, clofarabine and troxacitabine are undergoing clinical evaluation and show promising activity.

16. Synthesis of 1-(2-deoxy-2-isocyano-beta-D-arabinofuranosyl)cytosine and related nucleosides as potential antitumor agents; **J Med Chem,** December 24, 1993; 36(26): 4190-4; A. Matsuda, A. Dan, N. Minakawa, S.J. Tregear, S. Okazaki, Y. Sugimoto and T. Sasaki; Nucleosides and nucleotides. 123.

2'-Deoxy-2'-isocyano-1-beta-D-arabinofuranosylcytosine (8, NCDAC) has been synthesized as a potential antitumor **antimetabolite** from a corresponding 2'-azido-2'-deoxy-1-beta-D-arabinofuranosyluracil derivative 2a. Uracil and thymine analogues 6a and 6b of 8 were also prepared. Attempts to synthesize 2'-deoxy-2'-isocyanocytidine (14b) failed due to the insertion of the 2'-alpha isocyano group into the 3'-OH group, affording the 2',3'-oxazoline derivative 15b. Stability of the isocyano derivative 6a and 2',3'-oxazoline derivative 15a under basic and acidic conditions were examined. The isocyano group in 6a was stable in basic conditions but unstable

even in weakly acidic conditions to furnish the corresponding 2'-beta formamide derivative 17. Compound 15a was easily hydrolyzed the corresponding 2'-alpha formamide derivative 16 on treatment with H2O at room temperature. The cytotoxicity of 8, 6a, and 6b was examined in mouse and human tumor cells in vitro and compared with that of ara-C. Of these **nucleoside**s, 8 was moderately cytotoxic to these cell lines. In vivo antitumor activity of 8 against Lewis lung carcinoma cells was also investigated and 8 showed only moderate tumor volume inhibition.

17. Nucleosides as Antimetabolites: Thioguanine, mercaptopurine: their analogs and nucleosides as antimetabolites.**Curr Pharm Des,** January 1, 2003; 9(31): 2627-42; G.H. Elgemeie

Mercaptopurine (6MP) and 6-thioguanine (6TG) are analogs of the natural purines: hypoxanthine and guanine. Both mercaptopurine and thioguanine are substrates for hypoxanthine-guanine phosphoribosyltransferase and are converted into the ribonucleotides 6-thioguanosine monophosphate (6-thioGMP) and 6-thioinosine monophosphate (T-IMP) respectively. The accumulation of these monophosphates inhibits several vital metabolic reactions. Today, these thiopurine bases remain valuable agents for the induction and maintenance of remissions in patients with myelocytic and acute lymphocytic leukemia. Despite their proved clinical importance, 6MP and 6TG have certain therapeutic disadvantages, which have continued to stimulate the search for purine derivatives enhancing therapeutic efficacy. Considerable efforts have been made to prepare other novel mercaptopurine and thioguanine analogs and their **nucleoside**s to improve the antitumor efficacy. The effectiveness of these thiopurines against certain tumor cell lines suggested that some of these mercaptopurine analogs and their **nucleoside**s would be worthy of consideration in order to determine whether they exert a more selective effect against neoplastic cells than against normal cells or they might be useful in patients whose disease has become resistant to 6MP or 6TG. This review will focus on mercaptopurine analogs and their **nucleoside**s as **antimetabolite re**agents.

18. Metabolism of pyrimidine analogues and their nucleosides; **Pharmacol. Ther.,** January 1, 1990; 48(2): 189-222; G.C. Daher, B.E. Harris, and R.B. Diasio

The pyrimidine **antimetabolite** drugs consist of base and **nucleoside** analogues of the naturally occurring pyrimidines uracil, thymine and cytosine. As is typical of **antimetabolite**s, these drugs have a strong structural similarity to endogenous nucleic acid precursors. The structural differences are usually substitutions at one of the carbons in the pyrimidine ring itself or substitutions at on of the hydrogens attached to the ring of the pyrimidine or sugar (ribose or deoxyribose). Despite the differences noted above, these analogues, can still be taken up into cells and then metabolized via anabolic or catabolic pathways used by endogenous pyrimidines. Cytotoxicity results when the **antimetabolite** either is incorporated in place of the naturally occurring pyrimidine metabolite into a key molecule (such as RNA or DNA) or competes with the naturally occurring pyrimidine metabolite for a critical enzyme. There are four pyrimidine **antimetabolite**s that are currently used extensively in clinical oncology. These include the fluoropyrimidines fluorouracil and fluorodeoxyuridine, and the cytosine analogues, cytosine arabinoside and azacytidine.

19. Transport of Nucleoside antimetabolites in Cancer Cells; Nucleoside anticancer drugs: the role of nucleoside transporters in resistance to cancer chemotherapy; **Oncogene,** October 20, 2003; 22(47): 7524-36; V.L. Damaraju, S. Damaraju, J.D. Young, S.A. Baldwin, J. Mackey, M.B. Sawyer and C.E. Cass
antimetabolite into cells and outlines various possible mechanisms

The clinical efficacy of anticancer **nucleoside** drugs depends on a complex interplay of transporters mediating entry of **nucleoside** drugs into cells, efflux mechanisms that remove drugs from intracellular compartments and cellular metabolism to active metabolites. **Nucleoside** transporters (NTs) are important determinants for salvage of preformed **nucleoside**s and mediated uptake of **antimetabolite nucleoside**drugs into target cells. The focus of this review is the two families of human **nucleoside** transporters (hENTs, hCNTs) and their role in transport of cytotoxic chemotherapeutic **nucleoside** drugs. Resistance to anticancer **nucleoside** drugs is a major clinical problem in which NTs have been implicated. Single nucleotide polymorphisms (SNPs) in drug transporters may contribute to interindividual variation in response to **nucleoside** drugs. In this review, we give an overview of the functional and molecular characteristics of human NTs and their potential role in resistance to **nucleoside** drugs and discuss the potential use of genetic polymorphism analyses for NTs to address drug resistance.

20. Potential Multifunctional Antitumor Nucleosides and Analogues; 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)-cytosine, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil, and their nucleobase analogues as new potential multifunctional antitumor nucleosides with a broad spectrum of activity; **J. Med. Chem.,** December 6, 1996; 39(25): 5005-11; H. Hattori, M. Tanaka, M. Fukushima, T. Sasaki, and A. Matsuda; Nucleosides and nucleotides. 158.

We previously designed 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil (EUrd) as a potential multifunctional antitumor **nucleoside antimetabolite**. It showed a potent and broad spectrum of antitumor activity against various human tumor cells in vitro and in vivo. To determine the structure-activity relationship, various nucleobase analogues of EUrd, such as 5-fluorouracil, thymine, cytosine, 5-fluorocytosine, adenine, and guanine derivatives, were synthesized by condensation of 1-O-acetyl-2,3,5-tri-O-benzoyl-3-C-ethynyl-alpha,beta-D-ribo-pentofur anose (6) and the corresponding pertrimethylsilylated nucleobases in the presence of SnCl4 or TMSOTf as a Lewis acid in CH3CN followed by debenzoylation. The in vitro tumor cell growth inhibitory activity of these 3'-C-ethynyl **nucleoside**s against mouse leukemia L1210 and human nasopharyngeal KB cells showed that 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine (ECyd) and EUrd were the most potent inhibitors in the series, with IC50 values for L1210 cells of 0.016 and 0.13 microM and for KB cells of 0.028 and 0.029 microM, respectively. 5-Fluorocytosine, 5-fluorouracil, and adenine **nucleoside**s showed much lower activity, with IC50 values of 0.4-2.5 microM, while thymine and guanine **nucleoside**s did not exhibit any activity up to 300 microM. We next evaluated the tumor cell growth inhibitory activity of ECyd and EUrd against 36 human tumor

cell lines in vitro and found that they were highly effective against these cell lines with IC50 values in the nanomolar to micromolar range. These **nucleosides** have a similar inhibitory spectrum. The in vivo antitumor activities of ECyd and EUrd were compared to that of 5-fluorouracil against 11 human tumor xenografts including three stomach, three colon, two pancreas, one renal, one breast, and one bile duct cancers. ECyd and EUrd showed a potent tumor inhibition ratio (73-92% inhibition relative to the control) in 9 of 11 and 8 of 11 human tumors, respectively, when administered intravenously for 10 consecutive days at doses of 0.25 and 2.0 mg/kg, respectively, while 5-fluorouracil showed potent inhibitory activity against only one tumor. Such excellent antitumor activity suggests that ECyd and EUrd are worth evaluating further for use in the treatment of human cancers.

21. **Antitumor activity and pharmacokinetics of TAS-106, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine, Jpn. J. Cancer Res,** March 1, 2001; 92(3): 343-51, Y. Shimamoto, A. Fujioka, H. Kazuno, Y. Murakami, H. Ohshimo, T. Kato, A. Matsuda, T. Sasaki, and M. Fukushima.

We examined the effects of dosage schedule on antitumor activity in vitro and in vivo to determine the optimal administration schedule for a new **nucleoside antimetabolite** 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine (ECyd, TAS-106). The cytotoxicity of TAS-106 in vitro against human tumors was evaluated at three drug exposure periods. TAS-106 exhibited fairly potent cytotoxicity even with 4 h exposure, and nearly equivalent and sufficiently potent cytotoxicity with 24 and 72 h exposures. These results suggest that long-term exposure to TAS-106 will not be required to achieve maximal cytotoxicity. The antitumor activity of TAS-106 in vivo was compared in nude rat models bearing human tumors on three administration schedules, once weekly, 3 times weekly, and 5 times weekly for 2 or 4 consecutive weeks. TAS-106 showed strong antitumor activity without serious toxicity on all three schedules, but the antitumor activity showed no obvious schedule-dependency in these models. When tumor-bearing nude rats were given a single i.v. dose of [(3)H]TAS-106, tumor tissue radioactivity tended to remain high for longer periods of time as compared to the radioactivity in various normal tissues. Furthermore, when the metabolism of TAS-106 in the tumor was examined, it was found that TAS-106 nucleotides (including the active metabolite, the triphosphate of TAS-106) were retained at high concentrations for prolonged periods. These pharmacodynamic features of TAS-106 may explain the strong antitumor activity without serious toxicity, observed on intermittent administration schedules, in nude rat models with human tumors. We therefore consider TAS-106 to be a promising compound which merits further investigation in patients with solid tumors.

22. Combinations of 5-fluorouracil and N-(2-Chloroethyl)-N-nitrosourea moieties separated by a three-carbon chain; The synthesis of antitumor activity in mice of molecular combinations of 5-fluorouracil and N-(2-Chloroethyl)-N-nitrosourea moieties separated by a three-carbon chain; **J. Med. Chem.,** March 29, 1996; 39(7): 1403-12; R.S. McElhinney, J.E. McCormick, M.C. Bibby,

J.A. Double, M. Radacic, and P. Dumont; **Nucleoside analogs. 14.**

5-fluorouracil (5-FU) seco-nucleosides having as the "sugar" moiety a two-carbon (C2) side chain carrying a N-(2-chloroethyl)-N-nitrosourea group were designed as molecular combinations of **antimetabolite** and alkylating agent, but hydrolytic release of free 5-FU was not fast enough for significant contribution to the high activity they showed against colon and breast tumors in mice. In the present study of the synthesis of the more reactive C3 seco-**nucleoside**s, it emerged that, of various groups attached to the aldehydic center in the precursor phthalimides, only the alkoxy/uracil-1-yl type was conveniently obtained by the standard method. The methylthio/uracil-1-yl analog required relatively large amounts of reagent methanethiol, and exploration of alternatives involving alpha-chlorination of alkyl methyl sulfide or Pummerer rearrangement of its S-oxide, or successive hydrolysis and methylation of isothiouronium bromide, gave disappointing yields. For successful preparation of the alkoxy/uracil-3-yl compounds, the route used for C2 homologs required considerable experimental modification. In addition to these O,N- and S,N-acetals, some N,N-acetals bearing two 5-FU residues were prepared. The new drugs have been tested against a panel of experimental tumors in mice. Although it is evident from a parallel study that even these C3 seco-**nucleoside**s release free 5-FU too slowly in vivo, several of them have shown impressive anticancer activity. Reviewing their performance in comparison with earlier molecular combinations, a short list of seven [B.4152 (6), B.4015 (5), B.4030 (10), B.3999 (4), B.3995 (2), B.4083 (3), and B.3996 (the N 3-substituted analog of 1)] should be investigated further. This is particularly appropriate in light of the present understanding of the mode of action of chloroethylating agents. Following a prolonged period of clinical impatience with nitrosoureas because of limited selectivity action, a new era is confidently anticipated as these powerful drugs are increasingly studied in combination with O6-benzylguanine and other more efficient inhibitors of repair enzymes like O6-alkylguanine-DNA-alkyltransferase now being developed.

23. Modulation of the equilibrative nucleoside transporter by inhibitors of DNA synthesis; Br. J. Cancer, October 1, 1995; 72(4): 939-42; J. Pressacco, J.S. Wiley, G.P. Jamieson, C. Erlichman, and D.W. Hedley

Expression of the equilibrative, S-(p-nitrobenzyl)-6-thioinosine (NBMPR)-sensitive **nucleoside** transporter (es), a component of the **nucleoside** salvage pathway, was measured during unperturbed growth and following exposure to various **antimetabolite**s at growth-inhibitory concentrations. The probe 5-(SAENTA-x8)-fluorescein is a highly modified form of adenosine incorporating a fluorescein molecule. It binds. with high affinity and specificity to the (es) **nucleoside** transporter at a 1:1 stoichiometry, allowing reliable estimates of es expression by flow cytometry. Using a dual labelling technique which combined the vital DNA dye Hoechst-33342 and 5-(SAENTA-x8)-fluorescein, we found that surface expression of es approximately doubled between G1 and G2 + M phases of the cell cycle. To address the question of whether es expression could be modulated in cells exposed to drugs which inhibit de novo synthesis of nucleotides, cells were exposed to **antimetabolite** drugs having different modes of action.

Hydroxyurea and 5-fluorouracil (5-FU), which inhibit the de novo synthesis of DNA precursors, produced increases in the expression of es. In contrast, cytosine arabinoside (ara-C) and aphidicolin, which directly inhibit DNA synthesis, produced no significant increase in es expression. Thymidine (TdR), which is an allosteric inhibitor of ribonucleotide reductase that depletes dATP, dCTP and dGTP pools while repleting the dTTP pool, had no significant effect on es expression. These data suggest that surface expression of the es**nucleoside** transporter is regulated by a mechanism which is sensitive to the supply of deoxynucleotides. Because 5-FU (which specifically depletes dTTP pools) causes a large increase in expression whereas TdR (which depletes all precursors except dTTP) does not, this mechanism might be particularly sensitive to dTTP pools.

24. In **Vitro Cell Dev Biol; Br. J. Cancer,** October 1, 1995; 72(4): 939-42.
November 1, 1991; 27A (11): 873-7; M. Moorghen, P. Ince, K.J. Finney, A.J. Watson, and A.L. Harris, Department of Pathology, University of Newcastle upon Tyne, United Kingdom

The in-vitro effects of hydroxyurea , nucleoside antimetabolites 5-FU and 5-FUdR have been extensively studied in experimental systems employing cell-line techniques. In this study we investigated the effects of these drugs on the levels of incorporation of labeled **nucleoside**s into DNA in explants of intact rat colonic mucosa maintained in organ culture. The effects of the **nucleoside** transport inhibitors nitrobenzylthioinosine (NBMPR) and dipyridamole--which are modulators of **antimetabolite** cytotoxicity, on the incorporation of tritiated thymidine ([3H]TdR) into DNA were also studied. The incorporation of tritiated TdR into DNA was reduced by hydroxyurea but was not altered by either 5-FU or 5-FUdR. The levels of tritiated deoxyuridine were reduced by 5-FU and 5-FUdR in separate experiments; this is in keeping with thymidylate synthase inhibition. NBMPR and dipyridamole also reduced 3H-TdR incorporation into DNA. These results can be explained in terms of the known mechanisms of action of these drugs. This experimental model is therefore useful in assessing the effects of **antimetabolite**s and **nucleoside** transport inhibitors in intact colonic mucosa.

25. Potentiation of the cytotoxicity of thymidylate synthase (TS) inhibitors by dipyridamole analogues with reduced alpha1-acid glycoprotein binding.
Br. J. Cancer, August 1, 1999; 80(11): 1738-46; N.J. Curtin, K.J .Bowman, R.N. Turner, B. Huang, P.J. Loughlin, A.H. Calvert, B.T. Golding, R.J. Griffin and D.R. Newell.

Dipyridamole has been shown to enhance the in vitro activity of **antimetabolite anticancer drugs** through the inhibition of **nucleoside** transport. However, the clinical potential of dipyridamole has not been realized because of the avid binding of the drug to the plasma protein alpha1-acid glycoprotein (AGP). Dipyridamole analogues that retain potent **nucleoside** transport inhibitory activity in the presence of AGP are described and their ability to enhance the growth inhibitory and cytotoxic effects of thymidylate synthase (TS) inhibitors has been evaluated. Three dipyridamole analogues (NU3026, NU3059 and NU3060) were shown to enhance the growth inhibitory activity of the TS inhibitor CB3717 and block thymidine rescue in L1210 cells. The extent of potentiation at

a fixed analogue concentration (10 microM) was related to the potency of inhibition of thymidine uptake. A further analogue, NU3076, was identified, which was more potent than dipyridamole with a Ki value for inhibition of thymidine uptake of 0.1 microM compared to 0.28 microM for dipyridamole. In marked contrast to dipyridamole, inhibition of thymidine uptake by NU3076 was not significantly affected by the presence of AGP (5 mg ml(-1)). NU3076 and dipyridamole produced equivalent potentiation of the cytotoxicity of the non-classical antifolate TS inhibitor, nolatrexed, in L1210 cells with both compounds significantly reducing the LC90, by > threefold in the absence of salvageable thymidine. Thymidine rescue of L1210 cells from nolatrexed cytotoxicity was partially blocked by both 1 microM NU3076 and 1 microM dipyridamole. NU3076 also caused a significant potentiation of FU cytotoxicity in L1210 cells. These studies demonstrate that **nucleoside** transport inhibition can be maintained in the absence of AGP binding with the dipyridamole pharmacophore and that such analogues can enhance the cytotoxicity of TS inhibitor.

26. Characterization of a multidrug resistant human **erythroleukemia cell line (K562) exhibiting spontaneous resistance to 1-beta-D-arabinofuranosylcytosine; Leukemia,**
S. Grant, A. Turner, P. Nelms and S. Yanovich; May 1, 1995; 9(5): 808-14.

We have assessed the response of a previously characterized multidrug resistant (MDR) human erythroleukemia cell line (K562R) to the **nucleoside** analog **antimetabolite** 1-beta-D-arabinofuranosylcytosine (ara-C). This cell line has been subjected to selection pressure by intermittent exposure to daunorubicin, but not ara-C, since its initial isolation. In comparison to the parental line (K562S), K562R were approximately 15-fold more resistant to ara-C as determined by 3H-dThd incorporation, MTT dye reduction and clonogenicity. Following a 4-h exposure to 10 microM ara-C, K562S accumulated approximately seven times more ara-CTP, and incorporated approximately 250% more ara-C into DNA than their resistant counterparts. The intracellular generation of ara-CTP was not significantly influenced by the cytidine deaminase inhibitor THU or the deoxycytidylate deaminase inhibitor dTHU (1 mM each) in either cell line. Rates of dephosphorylation of ara-CTP were equivalent in sensitive and resistant cells, as were intracellular levels of both ribonucleotide and deoxyribonucleotide triphosphates. However, K562R displayed a significant (ie 70%) reduction in the level of activity of the pyrimidine salvage pathway enzyme, deoxycytidine kinase (dCK), compared to K562S cells. In contrast to U937 leukemic cells, DNA extracted from K562S and K562R cells following exposure to 10 microM ara-C for 6 h did not exhibit the characteristic internucleosomal DNA cleavage on agarose gel electrophoresis typical of drug-induced apoptosis. Lastly, Northern analysis revealed equivalent levels of dCK message in the two cell lines. K562R represents an unusual example of a classical multidrug resistant human leukemic cell line exhibiting spontaneous cross-resistance to the **antimetabolite** ara-C, and may prove of value in attempts to understand the mechanism(s) by which human leukemic myeloblasts survive in vivo exposure to combination chemotherapeutic regimens containing drugs that are not classically associated with the multidrug resistance phenomenon.

27. Clofarabine: Bioenvision/ILEX; Curr Opin Investig Drugs,
A. Sternberg; December 1, 2003; 4(12): 1479-87;

Clofarabine is a purine **nucleoside antimetabolite** under development by Bioenvision (under license from the Southern Research Institute) and ILEX for the potential treatment of solid tumors, acute myelogenous leukemia, non-Hodgkin's lymphoma, and acute lymphoblastic and chronic lymphocytic leukemia. In September 2003, Bioenvision initiated a phase II trial in Europe in pediatric acute lymphoblastic leukemia, and in October 2003, ILEX submitted the first part of a rolling NDA to the FDA for the treatment of acute leukemia in children.

28. Corticosteroid responsive fludarabine pulmonary toxicity, Am. J. Clin. Oncol., August 1, 2002; 25(4): 340-1, G.S. Stoica, H.E. Greenberg and L.J. Rossoff.
Division of Pulmonary and Critical Care Medicine, Long Island Jewish Medical Center, New Hyde Park, New York 11042-1101, U.S.A

Fludarabine monophosphate is a purine **nucleoside antimetabolite** with efficacy in the treatment of lymphoproliferative disorders and chronic lymphocytic leukemia. It is the 2-fluoro, 5' phosphate derivative of 9-beta-D-arabinofuranosyl adenine (ara-A, vidarabine) and the mechanism of action is through inhibition of DNA synthesis and the cytolytic effects through the induction of endonuclease-independent apoptosis.

29. Cerebrospinal fluid pharmacokinetics and **toxicology of intraventricular and intrathecal arabinosyl-5-azacytosine** (fazarabine, NSC 281272) in the nonhuman primate.**Invest New Drugs,** May 1, 1993; 11(2-3): 135-40, R.L. Heideman, C. McCully, F.M. Balis and D.G. Poplack.

Arabinosyl-5-azacytosine (AAC), a new **nucleoside antimetabolite**, is broadly active in preclinical tumor screening evaluations. To assess the potential for intrathecal use of this drug, we studied the toxicity and pharmacokinetics of intrathecal and intraventricular administration in nonhuman primates. Four adult male rhesus monkeys were given single 10 mg intrathecal (n = 1) or intraventricular (n = 3) doses of AAC to determine its acute toxicity and pharmacokinetic parameters. An additional 3 animals were given four weekly 10 mg intrathecal doses to assess the systemic and neurologic toxicity associated with chronic administration. Disappearance from the cerebrospinal fluid (CSF) was biexponential, and CSF clearance was 0.2 ml/min, which exceeds the rate of CSF bulk flow by 5-fold. The peak CSF concentration and area under the concentration x time curve achieved with the intraventricular administration of 10 mg were one hundred, and fifty fold greater, respectively, than those achieved after an intravenous dose of 200 mg/kg (1500-2400 mg) in prior experiments. No clinically evident neurotoxicity was observed in either the single or the weekly x 4 dose groups. A slight, transient CSF pleocytosis and increased CSF protein was observed. Systemic toxicity was limited to one animal in the weekly x 4 dose group who demonstrated a mild and transient decrease in his peripheral leukocyte count unassociated with a change in his hematocrit or platelet count. These studies in nonhuman primates demonstrate a clear

pharmacokinetic advantage for intrathecal vs systemic administration of AAC. This is demonstrated by a 50-fold greater CSF drug exposure with an intrathecal or intraventricular dose 1/200th of that which can be given systemically.(ABSTRACT TRUNCATED AT 250 WORDS).

30. Phase I trial and biochemical evaluation of tiazofurin administered on a weekly schedule, **Sel. Cancer Ther.,** March 1, 1990; 6(1): 51-61, T.J. Melink, G. Sarosy, A.R. Hanauske, J.L. Phillips, J.H. Bayne, M.R. Grever, H.N. Jayaram and D.D. Von Hoff.

Tiazofurin (2-B-D-Ribofuranosylthiazole-4-Carboxamide: NSC 286193) is a **nucleoside antimetabolite** that acts as a potent inhibitor of IMP dehydrogenase resulting in a guanine nucleotide deprivation. Recent in vivo biochemical observations in rats bearing hepatoma suggested a correlation between depletion of guanine nucleotides and antitumor effect. The present phase I trial utilized a weekly x 3 bolus infusion schedule, repeated every 5 weeks. Biochemical measurements of GTP and dGTP were performed in patients at each dose level. Twelve patients received 16 courses of the drug in doses ranging from 1100 to 2050 mg/m2 weekly x 3. The dose limiting toxicities were pericarditis and clinical symptoms suggestive of a more generalized serositis (chest and abdominal pain). Other toxicities included reversible elevations in CPK (MM band only) and SGOT, nausea, vomiting, and arthralgias. Neurotoxic effects were generally mild, including headaches, anxiety, and malaise. Only 1 of 6 patients evaluated for tiazofurin's biochemical activity showed a sustained depletion of guanine nucleotide pools. No antitumor activity was observed. The maximally tolerated dose of tiazofurin on this intermittent weekly x 3 schedule was 1650 mg/m2. Toxicity and the overall lack of biochemical and biologic effect at clinically achievable doses may preclude further clinical evaluation of this drug on a weekly schedule. The toxicities observed in our study were similar to those reported for phase I investigations using a considerably higher dose intensity with daily x 5 schedules.

31. Evaluation of purine and pyrimidine analogues in human tumor cells from patients with low-grade lymphoproliferative disorders using the FMCA, **Eur. J. Haematol,** May 1, 1999; 62(5): 293-9, Aleskog, R Larsson, M Hoglund, C Sundstrom, and J Kristensen.

The purine analogues fludarabine and cladribine (CdA) have recently become established to be effective treatment for low-grade non-Hodgkin's lymphoma (NHL). The pyrimidine **nucleoside** analogue cytarabine (AraC) has an important place in the treatment of acute leukemia, and gemcitabine is a new **pyrimidine antimetabolite** which has shown clinical activity against solid tumors. We have used the semiautomated fluorometric microculture cytotoxicity assay (FMCA), based on the measurement of fluorescence generated from cellular hydrolysis of fluorescein diacetate (FDA), to study these drugs. Eighty samples from 60 patients with low-grade NHL were studied. Fifty samples from patients with acute lymphoid leukemia (ALL) and 118 samples from patients with acute myeloid leukemia (AML) were included for comparison. The results indicate that the purine- and pyrimidine **nucleoside** analogues tested may be as active against low-grade NHL as against acute leukemia. In low-grade NHL, AraC seems to be even more

active in comparison to CdA (p=<0.0001) and fludarabine (p=0.001). Untreated patients were more drug sensitive than previously treated patients. Gemcitabine showed the highest correlation with AraC (0.90) whereas CdA showed the highest correlation with fludarabine (0.84). Based on these results we propose that AraC and gemcitabine may have a role in the treatment of low-grade NHL.

32. Altered susceptibility of differentiating HL-60 cells to apoptosis induced by antitumor drugs, Leukemia, February 1, 1994; 8(2): 281-8, G Del Bino, X. Li, F. Traganos, and Z. Darzynkiewicz.

It has been reported that human promyelocytic leukemic HL-60 cells which undergo differentiation fail to respond by apoptosis when treated with antitumor drugs, predominantly DNA topoisomerase inhibitors. Because S phase cells are selectively sensitive to these drugs, and during differentiation there is a reduction in the proportion of cells in S phase, the reported decrease in the number of apoptotic cells could simply be a reflection of the paucity of sensitive cells in these cultures. Using cytometric methods which allow apoptosis to be related to cell cycle position, we have compared the apoptotic response of HL-60 cells growing exponentially and induced to myeloid differentiation by dimethyl sulfoxide (DMSO). The cells were treated with: (i) the DNA topoisomerase I inhibitor camptothecin (CAM), which selectively triggers apoptosis or S phase cells; (ii) the **nucleoside antimetabolite** 5-azacytidine (AZC) and hyperthermia, both of which preferentially affects G1 cells; and (iii) gamma radiation, which causes apoptosis predominantly of G2 + M cells. The cells exposed to 1.4% DMSO for 24 or 48 h were significantly more resistant to response by apoptosis, regardless of the nature of the agent and regardless of their position in the cell cycle. Thus, induction of differentiation lowers the cell's ability to respond to a variety of damaging agents by apoptosis and this effect is not correlated with cell cycle position. In addition, the difference in response was unrelated to expression of the apoptosis-modulating protein bcl-2, which appeared unchanged following 48 h exposure to DMSO. On the other hand, when the cells were pretreated with low concentrations of CAM or AZC, washed free of drug, and then treated with DMSO, the proportion of cells undergoing apoptosis was markedly increased, relative to drug-treated cells returned to DMSO-free medium. The present data may indicate that while the drug-induced damage screening mechanisms, which are linked to triggering apoptosis, may be more proficient in proliferating cells, the effectors of apoptosis are more expressed in cells undergoing differentiation. The data also suggest that the efficiency of chemotherapeutic agents or radiation may be reduced if a differentiating agent is used in combination therapy and is administered first. An enhancement of apoptosis, however, may be expected if the differentiating drug is administered in the reverse sequence.

33. Polarographic properties and **potential carcinogenicity of some natural nucleosides and their synthetic analogues**; **Bioelectrochem Bioenerg,** February 1, 1999; 48(1): 129-34.; L. Novotny, A.Vachalkova, and A Piskala

The polarographic reduction and the index of potential carcinogenicity tg alpha determined polarographically in aprotic conditions and in the presence of alpha-lipoic acid of nine naturally occurring and synthetic pyrimidine and six synthetic 1,3,5-triazine (5-aza) **nucleosides** was compared to the reduction of eight synthetic 1,3,6-triazine (6-aza) **nucleosides**. **Nucleosides** are of interest because of their key role in the nucleic acid structure and because of the **antimetabolite** and cytotoxic/antileukemia properties of their synthetic analogues. It was shown that polarographic reduction of the studied compounds is achieved at gradually increased potentials in the order of 6-aza < 5-aza < **pyrimidine nucleosides**. On other hand, the potential carcinogenicity of studied compounds increases usually in the order of pyrimidine < 6-aza << 5-aza **nucleoside**. The only compounds with remarkable potential carcinogenicity identified at this study were those ones from the 5-aza (1,3,5-triazine) **antimetabolite** series-arabinosyl-5-azacytosine (0.275), 5-aza-cytidine (0.295) and 5-aza-uracil (0.400)-and 2,2'-anhydrouridine (0.260). The relation of the data obtained to biological activity of **nucleosides** included in the study is discussed.

34. The Following Articles were taken from the book" **Drug Resistance and Selectivity, Biochemical and Cellular Basis , Edited by Enrico Mihich, Roswell Park Memorial Cancer Institute, Buffalo, NY,; Academic Press, 1973, Pages 83-93,** Chapter 3. CROSS-RESISTANCE AND COLLATERAL SENSITIVITY; Dorris J. Hutchinson and Franz A. Schmid

IV. Purine Analogs

Resistance to purine analogs was first described by Law and Boyle (1951) for 8—azaguanine in the L1210 mouse leukemia. As with all other chemo therapeutically useful antitumor drugs, neoplasms and other model systems resistant to 6-MP were described (Hutchison, 1963) shortly after the observed activity in experimental systems. On the biochemical level, the early studies on purine antagonist-resistant biological systems have been reviewed and summarized by Brockman (1963a,b) and Balis (1968). Un-doubtedly comparative studies on purine analog-resistant mutants and their wild-type parental lines have provided more information basic to the understanding of purine biosynthesis and metabolism than if only wild-type systems had been available.

Of the various mechanisms of resistance to purine analogs the most common
was decreased or deleted enzymatic capacity to convert the analogs the analog to a nucleotide, the actual biologically active purine derivative. If, however, the pyrophosphorylase is functional but to a lesser extent than in the wild type (missed population of cells, less active enzymatic protein, etc.), some degree of response will be seen in the system to related compounds. Other mechanisms have been tabulated (Hutchison, 1963, 1965) and the more common are discussed in chapter 7.

Animal neoplasms resistant to 6-MP, thioguanine, and 6-methyl thio- purine ribonucleoside (6-MeMPR), which have been targets for chemotherapy studies and not summarized by us previously, are listed in Table VI.

Line L1210/MP(III) reported (Hutchison *et al.,* 1962) to be collaterally sensitive to methotrexate, azaserine, and mitomycin C shows collateral sensitivity to the antibiotic, neocarzinostatin, to the alkylating agent, carbazilquinone, and to three new antifolates. It retained sensitivity to 6-MeMPR and ara-C.

Rutman et al. (1962) and Rutman (1964), who used a thioguanine resistant variant of L1210, reported collateral sensitivity to six alkylating agents but no change in response to cytoxan. Unlike L1210/MP(III), the sensitivity of L1210/TG/R to methotrexate and azaserine was not altered- it remained the same as the parental line.

Paterson and his group (Caldwell *et al.,*1967; Wang *et al.,* 1967; Paterson and Wang, 1970) found that an Ehrlich ascites resistant to either 6-MP or thioguanine was partially cross-resistant to 6-MeMPR. Likewise a 6-MeMPR-resistant Ehrlich ascites was partially cross-resistant to 6-MP. These observations of what can be called partial cross-resistance (the drugs, 6-MP or 6-MeMPR, appear to be twice as effective in the treatment of the wild-type Ehrlich ascites or against the several resistant lines) fit with the biochemical data (Wang *et al.,*1967; Paterson and Wang, 1970).

The chemotherapeutic results were similar when two thioguanine- resistant Ehrlich ascites lines were treated with 6-MeM PR (Table VI).

The expression of partial cross-resistant of thioguanine- and 6-MP-resistant lines to 6-MP- resistant lines to 6-MeMPR can be attributed to the fact that these lines were able to convert, enzymatically, 6-MeMPR to 6-MeMPR-5'-monophosphate. However, the 6-MeMPR-resistant line was capable of enzymatically forming some 6-MP ribonucleotide. The chemotherapeutic data and relative biochemical activities of the various purine analogs resistant to Ehrlich ascites are compatible.

V . Pyrimidine Analogs

Resistance **to a fluoropyrimidine** was first reported by Heidelberger *et al.* (1958). Many animal neoplasms and other biological systems that are resistant to fluoropyrimidines have been described since then (Hutchison,1963, 1965). In

general, they were all cross-resistant to other fluoro-pyrimidine analogs but retained their sensitivity to antifolates, purine analogs, and alkylating agents.

Rutman et al. (1962) and Rutman (1964) conducted extensive chemotherapy experiments using a 5-fluorouracil-resistant P815 neoplasm (P815-E176) with the specific purpose of searching for collateral sensitivity to alkylating agents and **antimetabolites**. Results of these tests are summarized in Table VII. Increased sensitivity was observed to five alkylating agents, three of which were newly syntlesized Compounds, but there was cross-resistance to triethylenethiophosphoramide

(thio-TEPA) and no change in response to the **antimetabolites,** cytoxan and nitrogen mustard (HN2). Rutman (1964) concluded that collateral sentivitity need not arise as an "all or none" phenomenon, i.e., there was no predictable pattern of response to a group of alkylating agents.

With continued interest in **pyrimidine analogs** and the resulting synthesis of ara-C (Walwick et al., 1959) and 1β-D-arabinofuranosyl-5-fluorocytosine (ara-FC) (Fox et al., 1966), Burchenal et al. (1966) found that a line of P815 resistant to 5-fluorouracil (FU) retained the same sensitivity as the P815 parent line to both cytosine analogs.

Heidelberger and Anderson (1964) described the antitumor activity of **5-trifiu roniethyl-2'-deoxyuridine (F_3TdR) against several animal neo**plasms including an Ehrlieh ascites resistant to 5-fiuorodeoxyuridine (FUdR). The resistant neoplasm was found to be cross-resistant to F_3TdR. Based on information relating to the mode of action of **FUdR** and bio- chemical alterations in the FUdR—resistant Ehrlich ascites, it was concluded that the inhibition of thymidylate synthetase may be more important in the mechanism of tumor inhibition than in the **incorporation of** the **analog into DNA.**

The mechanism of resistance to **fluorinated pyrimidines** and the history of their development have been summarized (Hutchison, 1963, 1965). Recent observations have added little to earlier results.

An interesting study was described by Blair and Hall (1969) in which they followed the development of resistance in the Ehrlich ascites to 6-azauracil and 6-azauridine. However, they were unable to correlate either decreased or increased activities of uridine kinase or uridine phosphorylase with development of resistance. One 6-azauracil-resistant line was cross-resistant to 6-azauridine and collaterally sensitive to FU (Table VII).

As mentioned, the attention of several laboratories was turned to cytosine derivatives. Vesely and his colleagues (1968, 1970) characterized two lines of the AKR mouse leukemia- one resistant to 5-azacytidine (AKR/r-AzCR) and the other to 5-aza-2'-deoxycytidine (AKR/r-AzCdR). The subline resistant to 5-azacytidine (AzCR) was cross-resistant to 5-aza-2'-deoxycytidine **(AzCdR) and ara-C.** This can be explained on the basis of a deletion or loss of the enzyme deoxycytidine kinase. On the other hand the subiine resistant to **AzCdR** was sensitive to AzCR but cross-resistant to ara-C. In this line uridine kinase functioned Normally and ribonucleic acid (RNA) polymerase activity increased. The cross-resistance to ara-C is probably due to the partial loss of deoxycytidine kinase.

1-β-D-Arabinofuranosylcytosine was synthesized in 1959 by the Upjohn group (Walwick et *al.,* 1959). Smith (1967) reviewed in detail the background and development of this interesting **pyrimidine analog.** Numerous biochemical studies have been carried out that used **ara-C as the antimetabolite** and others that used ara-C-resistant cell cultures and animal neoplasms. Clinical

results with ara-C in cases of acute lymphocytic and acute granulocytic leukemias have been favorable (Howard *et al.,* 1966; Ellison *et al.,* 1968).

Wodinsky and Kensler (1964) reported the selection of an ara-C-resistance subline of the L1210 mouse leukemia. Although resistance was not achieved in three transplant generations, it was complete at the tenth generation. A selected group of twenty-four drugs with diverse modes of action was tested against L1210 and L1210/ara-C. As indicated in Table VII cross-resistance was not observed to any compound and likewise no increase in sensitivity . That there was no cross-resistence to alkylating agents nitrosoureas, **purine and** pyrimidine analogs, nor antifolates was thought to be significant in regard to clinical use. A report (Evans *et al.,* 1964) had shown that a line of L1210/C95 resistant to methotrexate, 6-MP and cvtoxan was sensitive to ara-C (Table V).

Dixon and Adamson (1965) stated that ara_C-resistant variants of the L1210 mouse leukemia could be selected in one generation. This observation is quite different from that of Wodinsky and Kensler (1964) . One of the ara-C-resistant variants was compared with L1210 in respect to response to several known antileukemic drugs (Table VII). No cross-resistance nor collateral sensitivity was observed.

Several other ara-C-resistant neoplasms have been selected (Table VII), and no cross-resistance has been observed to compounds such as hydroxy urea, guanazole, pyridine-2-carboxaldehye thiosemicarhazone (TSC) , bis(guanyihydrazones) , and carbazilquinone. Line L51787/ara-C (Schmid and Hutchison, 1971b) was collaterally sensitive to L-asparaginase which is ill keeping with the noted increased sensitivity of L5178Y/CA55 to L-asparaginase (Schmid arid Hutchison, 197lb)

The **ara-C** was active against hydroxyurea-, vincristinetine-, VLB-, TSC-, cortisone- and methyiglyoxal bis(guanylhydrazone) (MGGH)-resistant
mouse leukemias (see Tables XXIII and XXIV). An L1210 line resistant to MGGH was collaterally sensitive to ara-C (see Table XXIII).

The mechanisms of resistance to ara-C have been reviewed and summarized by Uchida and Kreis (1969) and by Drahovsky and Kreis (1970) and are also discussed in Chapter 7. However, the overall observation of lack of cross-resistance in ara-C-resistant tumors and, conversely, no cross-resistance development in a variety of neoplasms resistant to other effective antileukemic drugs places ara-C in a favorable position as an effective chemotherapeutic agent at many stages or steps during the use of cyclic chemotherapy in the clinic.

Table VI : Animal Neoplasms Resistant to Purine Analogs

Response to chemotherapeutic drugs[a]

Drug	Neoplasm	same as parent line	Cross-resistant	collaterally sensitive	References
6-Mercaptopurine	Sarcoma 180 L1210/MP(III)	Ara-C 6-MeMPR Ara-C	-	Quinaspar Chlorasquin Methasquin Carbazilquinone Neocarzinostatin	Evans et.,1964 Bennett et.,1965 Bradner and Hutchison, 1966 Hutchison, 1968a Schmid and Hutchison, 1971c
	Ehrlich ascites (EAC-R1)	-	6-MeMPR[b]	-	Wang et al., 1967 Paterson and Wang, 1970
	L1210/MP	DIC	NSC-82196	-	Wodinsky et al., 1968
Thioguanine	L1210 TG/R	Methotrexate azaserine Cytoxan	5-Fluorouracil A-139	Nitrogen mustard Thio-TEPA L-PAM No. 30020 No 30024 No. 30025 No. 30035	Rutman et al., 1962
	Ehrlich ascites (ETGRI)	-	6-MeMPR[b]	-	Paterson and Wang, 1970
	(ETGRII)	-	6-Mercaptopurine	-	Paterson and Wang, 1970
6-Methyl thiopurine ribonucleoside	Ehrlich ascites (EAC-R2)	-	Formycin 6-Mercaptopurine[b]	-	Caldwell et al., 1967 Wang et al.., 1967 Paterson and Wang, 1970

Table VII

ANIMAL NEOPLASMS RESISTANT TO PYRIMIDINE ANALOGS

Response to chemotherapeutic drugs[a]

Drug	Neoplasm	Same as parent line[b]	Cross-resistant	Collaterally	Reference
5-Fluorouracil	P815-E176	Methotrexate Azaserine Thioguanine Cytoxan Nitrogen mustard No. 30024	Thio-TEPA	L-PAM A-139 No. 30020 No. 30025 No. 30035	Rutman et al., 1962 Rutman,1964
	P815/FU	Ara-C Ara-FC	-	-	Burchenal et al., 1966
5-Fluorodeoxy-uridine	Ehrlich ascites	-	5-Fluorouracil F_3TdR	-	Heidelberger and Anderson, 1964
6-Azauracil (AZU 1)	Ehrlich ascites	-	6-Azauridine	5-Fluorouracil	Blair and Hall, 1969
5-Azacytidine	AKR/r-AzCR	-	AzCdR Ara-C	-	Vesely et al., 1968 Vesely et al., 1970
5-Aza-2'-deoxy-cytidine	AKR-r-AzCdr	AzCR	Ara-C	-	Vesely et al., 1968, 1970
1β-D-Arabino-furanosylcyto-sine	L1210	Alkylating agents (3) Nitrosoureas (3) Phthalanilides (3) Purine antagonists (3) Pyrimidine Antagonists (3) Antifolates (2) Miscellaneous	-	-	Wodinsky and Kensler, 1964

	Compounds (7)			
L1210/CA	Hydroxyurea	-	-	Dixon and Adamson
	Hydroxycarbamic		1965	
	Acid ethyl ester			
	DCM			
	MGGH			
	BCNU			
P815	Hudroxyurea	-		-Kreis and Hutchison,
				1969
L1210-ara-C	MGGH		-	-Mihich et al., 1970
	DDUG			
L1210/ara-C	Hydroxyurea	-		-Schabel et al., 1971
	Guanazole			
TSC				
L5178Y/ara-C	Methotrexate	-		
		L-Asparaginase		Schmid and
				Hutchinson, 1971 b,c
	Cytoxan			
	Carbazilquinone			

Notes to Tables:

a;α Ara-C-1β-D-arabionfuranosylcytosine; are-FC-1β-arabionfuranosyl-5-fluorocytosine; No. 30024-1-bis(β-chlroethyl)amino-2-dimethyl aminoethane; DCM-3', 5'-dichloromethopterin; MGGH-methylglyoxal bis(guanylhydrazone); DDUG-4' , 4' – diacetyldipenylurea bis(guanylhydrazone); BCNU-1,3-bis(2-chloroethyl)-1-nitrosourea; TSC-pyridine-2-carboxaldehyde thiosemicarbazone; thio-TEPA-triethylenethiophosphoramide; F3TdR-5-trifluoromethyl-2'-deoxyuridine; AzCdR-5-aza-2'-deoxycytidine; L-PAM-L-phenylalanine mustard methanol; A-139-2, 5-bis(1-aziridinyl)-3,6-bis(2-methoxyethoxy)-p-benzoquinone; No. 30020-6-hydroxy-9-{3-[bis(2"-chloroethyl)amino}purine; No. 30025-1bis(β-chloroethyl)amino-4-aminopentane; No. 30035-1-bis(β-chloroethyl)amino-2-aminoethane).

[b]Numbers in parentheses indicate number of compounds in each group. "